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Acute and subacute toxicity of trichlorfon in guppies (*Poecilia reticulata*)

Gang-Joon Heo[1], Gee-Wook Shin[2,*]

[1]*College of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Korea*
[2]*Bio-Safety Research Institute and College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea*
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Abstract : The aim of the present study was to determine the acute and subacute potential of trichlorfon in guppies (*Poecilia reticulatus*). We first defined the 24 h median tolerance limit (TLm_{24h}) of the fish to trichlorfon. Guppies were then treated with TLm_{24h} and 1/10 TLm_{24h} trichlorfon concentrations to evaluate if any histological alterations occurred. The TLm_{24h} value of trichlorfon was 11 ppm. This concentration resulted in acute toxicity to the gills and kidneys with edema, hyperplasia of the gill lamellae, and vacuolated degeneration and necrosis of renal tubular cells. In the case of subacute toxicity using a 10-fold dilution of the TLm_{24h} value (1.1 ppm), no behavioral changes, external lesions or histopathological changes were observed. Therefore, safe concentration of trichlorfon might be 1.1 ppm in guppy for controlling parasitic infections.

Keywords : guppy, histopathology, TLm_{24h}, toxicity, trichlorfon

Guppies (*Poecilia reticulata*) are one of the most popular pet fish species. However, this fish species is susceptible to parasite infection by various parasites including *Gyrodactylus*, *Ichthyophthirius*, and *Tetrahymena* [9, 11, 13, 16]. Trichlorfon (2,2,2-trichloro-1-hydroxyethyl phosphonate) is one of the most frequently-used organophosphate compounds for controlling parasites in fisheries. In aqueous solution, it is very rapidly hydrolyzed to dichlorvos (2,2-dichlorovinyl dimethyl phosphate), which is much more toxic to fish as it inhibits acetylcholinesterase (AChE) [10]. Inhibition of AChE by dichlorvos results in lethargy and reduced feeding by the fish [14, 15]. In addition, inhibition of AChE stimulates the production of oxygen free radicals by activation of the N-methyl-D-aspartic acid receptor, and large quantities of glutathione and esterase are consumed during detoxification [4, 12]. Therefore, fish exposed to trichlorfon could be damaged by oxidative stress, leading to cell death and necrosis. Although many researchers have reported the side-effects of trichlorfon in different fish species [2, 3, 6, 8, 17], there is limited information about its toxic effects in guppies, especially in terms of its histopathological effects. Herein, we examined histopathological lesions in guppies caused by trichlorfon for investigating safe concentration to control various parasitic infections.

Healthy adult guppies (3.0 ± 0.5 cm, 0.8 ± 0.2 g) were subjected to trichlorfon toxicity tests. Prior to testing, the fishes were acclimated in the laboratory for 1 week in a 120 L holding tank containing 100 L of dechlorinated tap water and were fed twice daily with TetraBits (Tetra GmbH, Germany). After fasting for 24 h, toxicity tests were performed using 25 L-glass tanks equipped with an air pump, filter, thermostat, and fluorescent lamp. During acclimation and test periods, the water temperature was maintained at 25 ± 1°C and photoperiod cycles were kept constant at 12 h light/ 12 h dark. The toxic effects of trichlorfon were examined using Neguvon (Bayer HealthCare, Korea), a 98%-pure source of trichlorfon. For acute and subacute toxicity tests in guppies, we firstly defined the 24 h median tolerance limit ($TLm_{24h} = LC_{50}$), as described previously by Doudoroff *et al*. [5]. Briefly, guppies were divided into 5 groups of 10 fish each. The fish groups were exposed to 0, 10, 20, 40, or 80 ppm of trichlorfon and were then observed for 24 h. Subsequently, several

*Corresponding author: Geewook Shin
College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea
[Tel: +82-63-270-3903, Fax: +82-63-270-3778, E-mail: shingw@chonbuk.ac.kr]

more tests were performed under a progressively narrower range of trichlorfon concentrations to more precisely determine the TLm_{24h} concentration. Acute toxicity tests were carried out in 20 guppies treated with the TLm_{24h} concentration for 1 day. Subacute toxicity was induced in 20 fish exposed to a 10-fold dilution of the TLm_{24h} concentration for 7 days. The test solution was renewed daily during the course of this test. In addition, all guppies were examined for abnormal behavior and external lesions. After each toxicity test, histopathological changes were examined by hematoxylin and eosin (H&E) staining. Briefly, live guppies were euthanized using MS-222 (tricaine methanesulfonate; Sigma, USA), sacrificed by medullar section, and fixed in 10% formalin for 24 h. The fixed bodies were dehydrated in graded concentrations of ethanol, following decalcification of the bone tissue by 8% formic acid. The dehydrated bodies were embedded in paraffin, cut longitudinally into 4 μm sections, and then stained with H&E. The stained tissues were examined under light microscopes. All the procedures in this study were approved by the ethics committee of Chungbuk National University.

The TLm_{24h} value of trichlorfon in guppies was 11 ppm. To induce acute toxicity in guppies, 20 guppies were immersed into a 25 L test tank containing this concentration of trichlorfon. During the test, behavioral changes were frequently observed; for example, loss of equilibration, slowness of opercula movement, insensitivity to threatening stimuli, and swimming on the water surface. In addition, the fish exposed to 11 ppm trichlorfon produced excessive mucus on the skin and gills. In contrast, no mortality or clinical signs were observed in the control group. Histopathological signs of trichlorfon toxicity were evident in the gills and kidneys of all individuals treated with 11 ppm trichlorfon. However, no histopathological changes were seen in any other organs examined. In the group exposed to the acute toxic concentration of trichlorfon, edema and fusion of the secondary lamella as well as severe hyperplasia and exfoliation of the lamella epithelial cells were frequently observed in the gills (in 18 of 20 fishes). However, blood congestion was rarely present in the gills of trichlorfon-treated fish (Fig. 1) (in 8 of 20 fishes). The experimental group showed large spaces between tubules and tissues in the kidneys, eosinophilic material within the renal tubular cavity, and vacuolar degeneration and loss of the nucleus in renal tubular cells (Fig. 2) (in 18 of 20 fishes). However, these histopathological lesions were not seen in the control group. On the other hand, subacute toxicity tests using 1.1 ppm trichlorfon did not result in significant behavioral changes, external lesions and histopathological lesions.

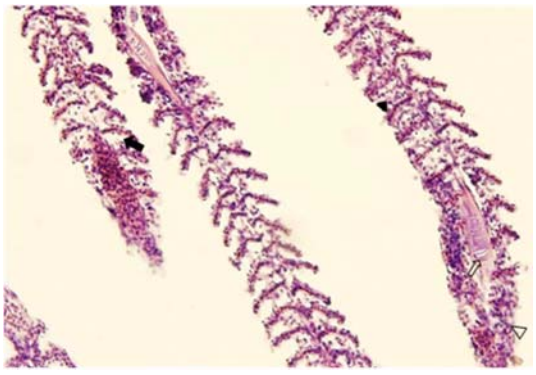

Fig. 1. Light photomicrograph of the gill of a guppy exposed to 11 ppm of trichlorfon for 24 h. H&E staining, ×200. The gill exhibited various histopathological changes, such as edema (black arrow) and fusion of the secondary lamella (open arrow head), hyperplasia of the lamella epithelial cell (black arrow head) and blood congestion (open arrow).

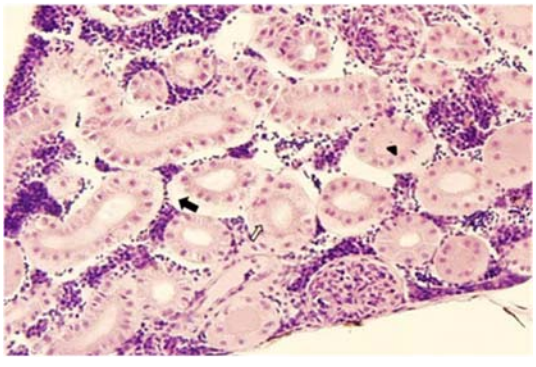

Fig. 2. Light photomicrograph of the kidney of a guppy exposed to 11 ppm of trichlorfon for 24 h. H&E staining, ×200. The kidney exhibited various histopathological changes, such as large spaces between tubules and tissues in the kidneys (black arrow), eosinophilic material within the renal tubular cavity (arrow head), and vacuolar degeneration and loss of the nucleus in renal tubular cells (open arrow).

In this study, we determined the TLm_{24h} value (LC_{50}) of trichlorfon in guppies. TLm_{24h} values for trichlorfon have been reported to be 26.5 ppm for the maya cichlid (*Cichlasoma urophthalmus* Günther) [7], 92 ppm for

medaka (*Oryzias latipes*) [17], and 5 ppm for the European eel (*Anguilla anguilla*) [6]. These previous studies suggest that the toxicity of trichlorfon differs among fish species [6, 7, 17]. Thus, compared to the previous studies [6, 7, 17], guppies are more sensitive to trichlorfon than the maya cichlid and medaka, but less sensitive than the European eel.

Fish gills, by virtue of their continuous contact with the water, have multiple functions including respiration, osmoregulation, acid-base balance, and nitrogenous water excretion. Therefore, this organ is highly sensitive to contaminants in the water. Histological changes on fish gills are therefore good indicators of water quality. Generally, a wide range of contaminants have common impacts on the gill, such as epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, and edema with epithelial lifting [1, 8]. Fish kidneys have several functions including hematopoiesis, osmoregulation, and the discharge of metabolites from the body. Kidneys also serve as a major route for excreting toxicants and their metabolites. Because renal tissues can potentially be continuously exposed to toxicants, non-specific histopathological lesions including degenerative changes in the tubular epithelium, dilation of the tubular lumina, cellular casts within the tubular lumina, tubular necrosis, and/or epithelial desquamation can be observed [1, 2]. In the present study, the histopathological changes observed in the gills and kidneys of guppy after exposure to acute toxic concentrations of trichlorfon were very similar to those reported in previous studies [1, 2, 8]. These gill and kidney lesions therefore appear to be non-specific symptoms that occur as a result of exposure to toxicants. Based on our study and those of others, we hypothesize that the toxic effects of trichlorfon in guppies are due to dysfunction of the gill and kidneys caused by the toxicity itself and/or oxidative stress [1, 4, 8, 12].

Significant histopathological lesions have been reported on the gills of *Oreochromis niloticus* after 4 h of post-exposure to 0.25 ppm of trichlorfon [8]. In addition, the kidney and liver of *Prochilodus lineatus* were damaged by concentrations of trichlorfon as low as 0.1 ppm [2, 3]. In contrast to previous studies [2, 3, 8], we observed no significant histological alterations in the tissues of fish exposed to subacutely toxic concentrations of trichlorfon (1.1 ppm) for 1 week. This may indicate that guppies have a higher natural resistance to trichlorfon than other fish species.

In summary, TLm_{24h} value for trichlorfon was determined in guppies. Administration of trichlorfon at the TLm_{24h} value to guppies resulted in lesions on the gills and kidneys of the guppies. However, a trichlorfon concentration 10-fold lower than the TLm_{24h} concentration did not cause significant histological alterations in the fish. Although further studies are needed for efficacies of trichlorfon to various parasitic infections in guppy, we recommend 1.1 ppm as available concentration of trichlorfon under present experimental condition.

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